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Resuspension of *E. coli* from Direct Fecal Deposits in Streams

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Abstract. Direct fecal deposits from cattle provide a significant source of E. coli *to streams and therefore pose a threat to human health in agricultural watersheds. Experiments were conducted in a flume (9.1 m long, 0.6 m wide, and 0.6 m deep) with flow of 0.0106* $m^3\ s^{-1}$*, an average velocity of 11.4 cm* s^{-1}*,and water depth of 15.24 cm to measure the resuspension and deposition of* E. coli *from an undisturbed standard cowpat. Water samples were collected 1.22 m and 3.66 m downstream of the deposited cowpat, and at each downstream cross-section nine samples were collected to characterize the bacterial movement.* E. coli *in water samples were separated into the attached and unattached phases by filtration to assess the mechanism of transport. The cumulative load contribution from a single deposited cowpat after one hour was* 2.49×10^9 *cfu 3.66 m downstream. The composite* E. coli *concentrations at all sampling points and times exceeded the federal standards for primary contact in the United States of 126 cfu/100 ml. Between 77.2 and 99.5% of all* E. coli *downstream of the direct deposit were associated with particulates. The resuspension rate was* 5.91×10^7 *and* 9.52×10^4 *cfu* $m^{-2}s^{-1}$ *0.5 min and 60 minutes after deposition, respectively, 1.22 m downstream of the deposit and* 2.19×10^6 *and* 3.14×10^3 *cfu* $m^{-2}s^{-1}$ *0.5 min and 60 min after deposition, respectively, 3.66 m downstream of the deposit. Results from this study are useful to improve modeling techniques to predict in-stream* E. coli *concentrations from direct fecal deposits and emphasize the need to implement management practices to reduce livestock access to streams.*

Keywords. Water quality, E. coli, *resuspension, direct fecal deposit, flume.*

Introduction

More than 40% of the assessed waters in the United States do not meet water quality standards including approximately 300,000 miles of rivers and shorelines and 5 million acres of lakes (U.S. EPA, 2009). The leading cause of these impairments in the United States is pollution due to harmful microorganisms. Predicting the risk to public health requires accurate models of the key processes: deposition, resuspension, transport, and survival in the sediment and the water column (Fries et al., 2006). Deposited cowpats have large *E. coli* concentrations, ranging from $4\text{x}10^6$ to $5\text{x}10^7$ cfu/g dry wt. (Soupir et al., 2008a), and the high nutrient concentrations and organic content provide an optimal environment to enhance fecal bacteria survival. Direct fecal deposits from cattle provide a significant source of pathogen indicators to streams in agricultural watersheds: for example, allowing cattle unrestricted access to streams in poorly drained Midwestern landscapes resulted in a 36-fold increase in stream *E. coli* concentrations (Vidon et al., 2008). Hall et al. (2007) identified reductions in direct fecal deposits as the leading change in management practices necessary to meet water quality standards in upland, rural Virginia watersheds.

Current watershed-scale models cannot properly account for the source of pathogen indicators from direct fecal deposits. For example, the only in-stream bacterial routing process currently included in the Soil and Water Assessment Tool is decay (Neitsch et al., 2005). Another model commonly used for Total Maximum Daily Load (TMDL) development, HSPF, also has limitations to its ability to simulate direct fecal deposits: During low or zero flow conditions, flow is continuously simulated; leading to erroneously elevated predicted in-stream bacteria concentrations (Hall et al., 2007). Current approaches for modeling direct fecal deposits during low flow conditions in HSPF include i) direct deposit stage cut-off, ii) flow stagnation, and iii) stream reach surface areas. Watershed scale models typically do not consider the timed release, deposition, or resuspension of direct fecal deposits.

Information is lacking on the release of pathogen indicators from direct fecal deposits as a function of flow rate. The objectives of this study are to i) assess *E. coli* release from direct fecal deposits and attachment to particulates over time, ii) calculate *E. coli* load contributions from a single direct deposit under various flows and water depths, and iii) measure *E. coli* resuspension rates from direct fecal deposits. Results from this study will result in improved understanding of in-stream release and transport of fecal indicators from direct fecal deposits.

Methods

Experiments were conducted in a Plexiglas flume (Figure 1) that is 9.1 m long, 0.6 m wide, and 0.6 m deep. The head gate and tail gate were adjusted to vary the depth of flow, and the discharge can be varied from 0 to 0.13 m^3s^{-1}. River rock was layered along the bottom of the flume to increase friction between the cowpat and the bottom surface and to make the flows more representative of a natural stream. A Nortek acoustic Doppler velocimeter was used to measure velocity at the two sampling cross sections, x = 1.22 m and x = 3.66 m (Figure 1), and a detailed velocity profile (10 points in the z direction and 5 transverse locations) was measured upstream of the fecal deposit. The discharge of 0.0106 $m^3 s^{-1}$ (10% uncertainty) was calculated from the detailed velocity profile. At the point where the cowpat was deposited into the flume, the velocity averaged 11.4 cm s^{-1} and the water depth was 15.24 cm. All measurements and sample collection occurred at steady flow, and samples were collected at 0.5 minutes, 15 minutes, 30 minutes, and 60 minutes following direct deposition. A grab sample was also collected during each sampling time at an upstream location, x = -1.86 m, to assess the background *E. coli* concentrations. Water leaving the flume is directed to a sump for recirculation. Tap water was de-chlorinated using Stress Coat (www.petmountain.com), and preliminary studies found no decrease in fecal indicator concentrations in tap water treated with Stress Coat when compared to fecal indicator concentrations in phosphate buffered solution (Soupir, 2009, unpublished data).

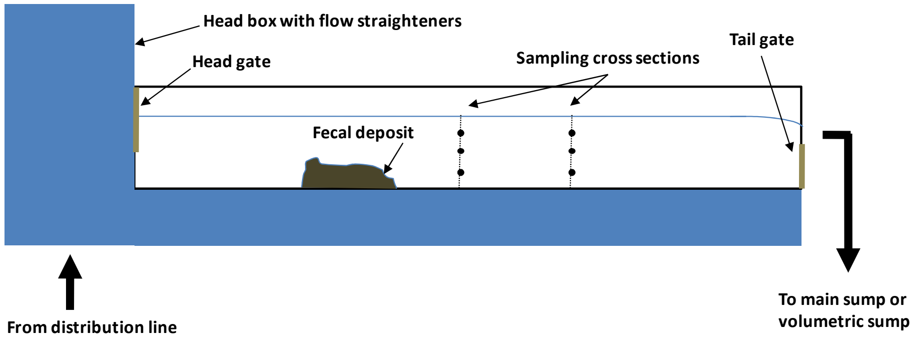

Figure 1. Schematic of the flume. Water samples were collected from 9 positions at the two sampling cross sections located at x = 1.22 and x = 3.66 m downstream of the fecal deposit.

Freshly excreted grazing beef cattle feces were collected from several animals at the ISU Beef Nutrition Research Farm. Standard cowpats were constructed following the procedures of Thelin and Gifford (1983). The feces were mixed, and the homogenized manure was placed in molds with a diameter of 20.3 cm and a depth of 2.54 cm until a weight of 0.9 kg was reached. Manure properties including *E. coli* concentrations, moisture content, temperature, nutrient concentrations, manure particle size distribution, bulk density, and organic matter content were determined in the Animal Waste Management and Water Quality Research Laboratories at ISU. Cowpat moisture content was adjusted to between 90 and 91% to represent freshly excreted feces.

E. coli in the manure and water samples was enumerated on modified mTEC agar (U.S. EPA, 2000) using membrane filtration techniques (APHA, 1998). Flows from each of the 9 points at a cross section were used to make a single flow-weighted composite sample from the two downstream sampling locations. The composite sample was analyzed for *E. coli* in the attached and unattached fractions. *E. coli* was separated into attached and unattached fractions based on a filter separation technique (8 µm nitrocellulose filter, Millipore) developed by Krometis et al. (2009) and dispersed using a hand shaker as described by Soupir et al. (2008b).

The load contribution to the water column over the one hour sampling period was calculated by the central flow-weighted method using the steady-state flow and the composite bacteria concentrations. The attached fraction was calculated as the difference between the total concentration and the unattached concentration for each composite sample. Resuspension rates were calculated with a mass balance applied between two sections:

$$R = \frac{Q(C_2 - C_1)}{A_S}$$

where R is the resuspension rate, C_1 and C_2 are the upstream and downstream composite *E. coli* concentrations, Q is the discharge, and A_S is surface area. For the upstream section, A_S is taken as the area of the flume covered by the direct fecal deposit, while for the downstream section it is taken as the bottom area between the two sampling sections.

Results and Discussion

In this section we present results from the first cowpat resuspension experiment, which was conducted at a flow of 0.0106 m^3s^{-1} and a depth of 15.24 cm. Additional experiments will be conducted and the results of these experiments will be available after analysis. Fresh manure concentrations averaged 3.89×10^5 (SD$\pm1.25\times10^4$) cfu/g (wet wt. basis) and falls within the range of fresh fecal material reported previously by Soupir et al. (2008).

Properties of the manure potentially influencing in-stream bacterial transport processes include moisture content, temperature, manure particle size distribution, bulk density, and organic content. The moisture content of representative fresh fecal samples collected from grazing beef cattle averaged 90.7%. Preliminary testing of the manure found that the moisture content decreased quickly between when the manure was collected in the field—immediately following excretion—and when the manure was added to the flume, approximately two hours later. Manure with a slightly higher moisture content (~90%) sank to the bottom of the flume, as would be expected of a direct deposit, based on field observation. Manure with lower moisture contents (typically measured approximately two hours after collection and ranged from 86.9% to 87.4%) floated along the surface of the water when added to the flowing water in the flume. For the experiments, we assumed initial manure moisture content of 87% and added 33 ml of distilled water per 100 grams of manure to increase the moisture content. The moisture content of the manure deposited into the flume was 91%, and the temperature was 24.5°C.

Deposition of the manure into the flowing water resulted in an initial plume of dispersed manure particles that quickly flowed downstream. Particles settled downstream of the direct deposit, and the area of observed fecal particles a few minutes after deposition measured 116.8 cm downstream of the initial deposition point and 38.1 cm in the transverse direction. Dispersed manure particles were still present in the rocks several hours after the last sample was collected (Figure 2).

Figure 2. Dispersed manure particles remaining on the bottom of the flume after the cessation of sampling.

Vertical profiles of bacteria release from direct deposits are shown in Figure 3. In general *E. coli* concentrations were greatest at the sampling point located closest to the rock layer and lowest at the sampling point closest to the water surface. During the 0.5 min sampling period, the bottom sampling point had 100% higher *E. coli* concentrations than the top sampling point at the 1.22 m downstream location but only 22% higher concentrations at the 3.66 m downstream location, which is attributed to vertical mixing in the water column. At later sampling times the percent differences between the bottom and top sampling locations decreased to 100%, 75%, and 48% at 15 minutes, 30 minutes, and 60 minutes after deposition, respectively at x = 1.22 m.

The recirculating nature of the flume quickly led to elevated background concentrations (Figure 4). Initially significant differences were observed in the samples downstream of the fecal deposit, but within 15 minutes background samples were nearly as high as the samples collected downstream of the deposit. At x = 1.22 m downstream from the direct deposit, the downstream sampling location was 98, 13, 16, and 30% higher than the background concentrations at the 0.5, 15, 30, and 60 minute sampling times, respectively. At x = 3.66 m downstream from the direct deposit, the downstream sampling location was 37, 0.7, 0.6, and 0.8% higher than the background concentrations at the 0.5, 15, 30, and 60 minute sampling times, respectively. Future experiments will examine *E. coli* concentrations under higher flows, and the higher velocities might increase resuspension after initial deposition (for example at times 15 – 60 minutes), resulting in greater differences between *E. coli* concentrations upstream and downstream of the cowpat. Also notable is the increase in concentrations at the 0.5 minute sampling time between the two sampling locations (x = 1.22 and x = 3.66 m downstream, Figure 4c). Based on the mean velocity of 11.4 cm s^{-1}, the peak in the plume passed the first sampling point (x = 1.22 m) at 11 seconds and the second sampling point (x = 3.66 m) at 32 seconds. Therefore, the plume or highest *E. coli* concentrations were captured at the downstream sampling location during the 0.5 minute sampling event. This observation is also supported by the higher load at the 3.66 m sampling point (Table 1) during the t = 0.5 min sample collection.

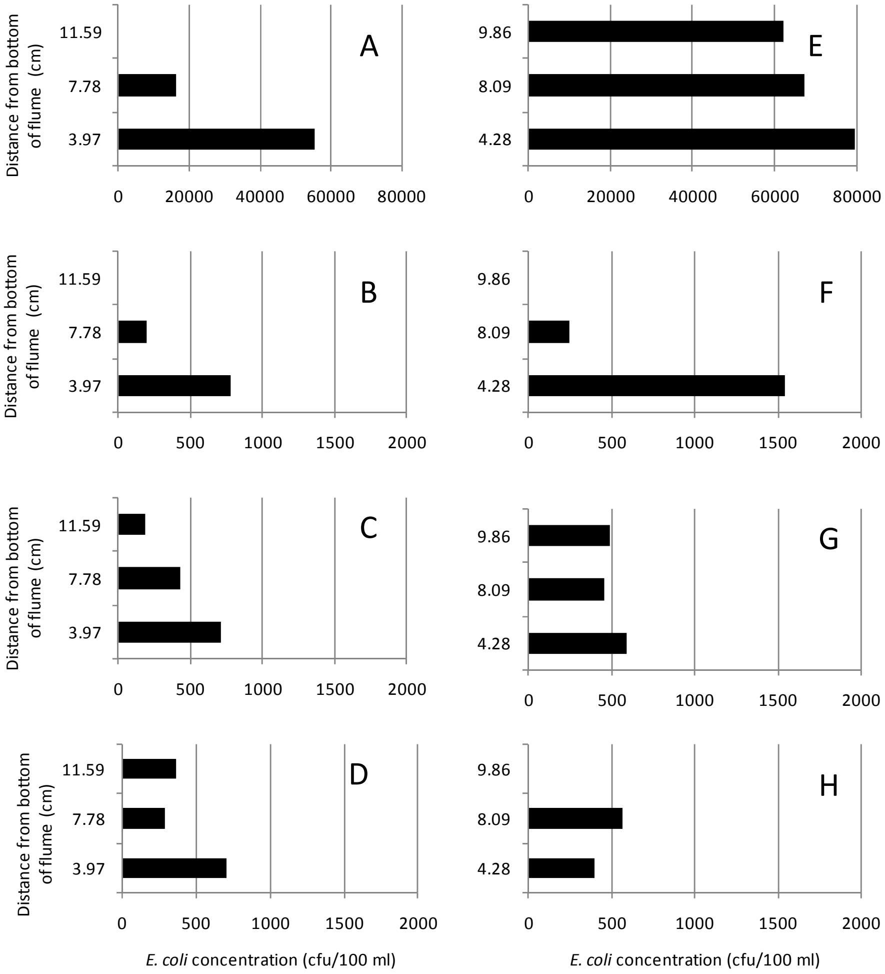

Figure 3. Vertical profiles of bacteria release from direct deposits with background contributions removed: A) 0.5 min sampling time at x = 1.22 m downstream from direct fecal deposit, B) 15 min sampling time at x = 1.22 m, C) 30 min sampling time at x = 1.22 m, D) 60 min sampling time at 1.22 m, E) 0.5 min sampling time at x = 3.66 m, F) 15 min sampling time at x = 3.66 m, G) 30 min sampling time at x = 3.66 m, H) 60 min sampling time at x = 3.66 m, a sample point is missing at the 9.86 cm distance.

The composite *E. coli* concentrations (adjusted for background concentrations) all exceed the federal standard for primary contact in the United States of 126 cfu/100 ml (Table 1). The one-hour cumulative load values describe the total contribution of *E. coli* to the water column from a direct fecal deposit during a one-hour sampling period. Additional contributions would be expected from the residual fecal material on the bottom of the flume (Figure 2), but the recirculation of the water supply made it difficult to assess additional contributions as background levels became elevated.

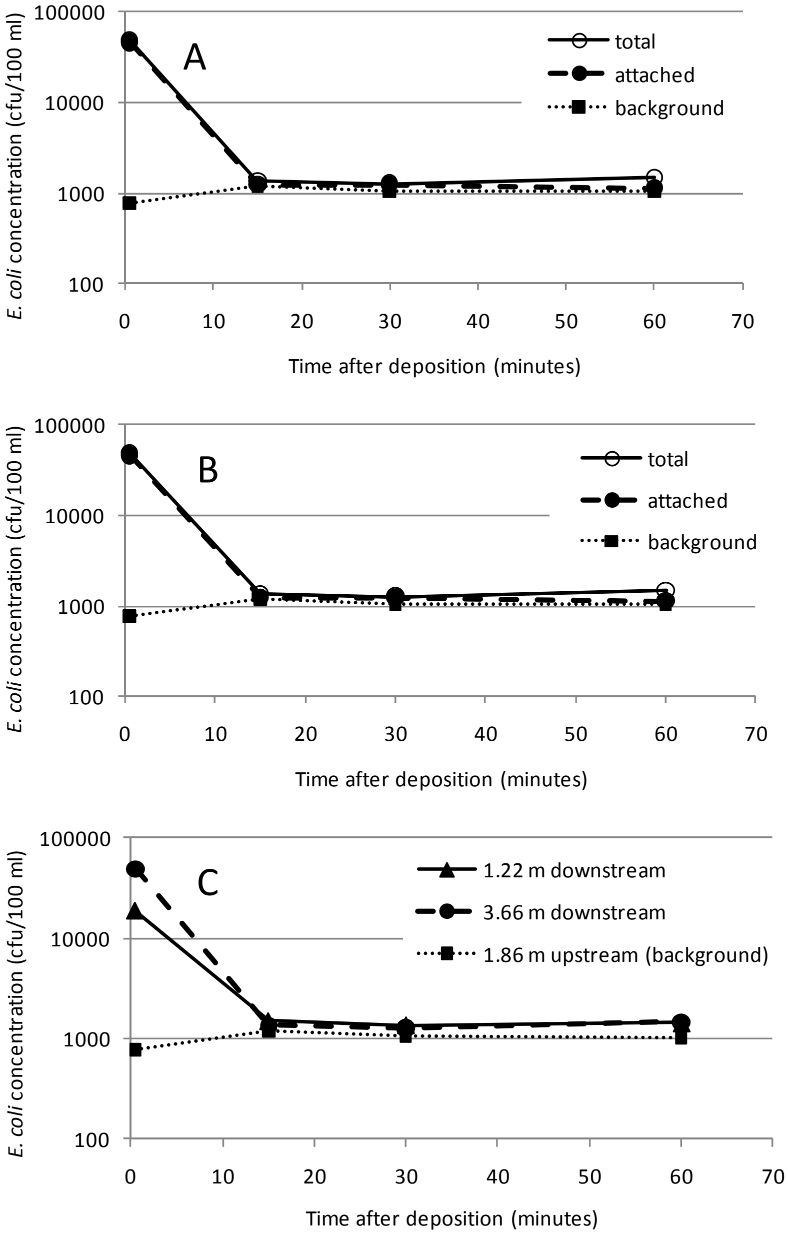

Figure 4. Time series of *E. coli* concentrations at A) x=1.22 m downstream of the direct deposit and B) x = 3.66 m downstream of the direct deposit. C) Comparison of total *E. coli* concentrations between the two sampling points and the upstream or background *E. coli* concentrations.

Resuspension rates provide an estimate of the movement of bacteria from the cowpat into the water column. The surface area contributing *E. coli* to the water column was assumed to be the size of a standard cowpat at t = 0.5 min at the 1.22 m sampling point and the area of the settled fecal material (0.445 m^2, Figure 2) at t = 15, 30, and 60 minutes. The area between the two sampling location (1.46 m^2) was used to calculate resuspension rates at the sampling point 3.66 m downstream of the cowpat. At t = 0.5 minutes, the resuspension rate at x = 1.22 m exceeded the resuspension rate at x = 3.66 by an order of magnitude. The resuspension at x = 1.22 is obviously attributed to the direct fecal deposit and the source of *E. coli* being resuspended at x = 3.66 during t = 0.5 minutes is attributed to manure particles that moved past the first sampling location between deposition and sample collection. Manure particles were observed to bounce along the rock layer and were likely deposited and resuspended many times while in the flume. As time progressed, the resuspension rates decreased (Table 1) at the first sampling point. At the second sampling point negative resuspension rates were observed, indicating deposition of fecal indicators in the stretch between the two sampling points. Resuspension rates of *E. coli* NAR inoculated in stream bottom sediments have been previously reported ranging from 8.2×10^3 and 1.1×10^4 cfu $m^{-2}s^{-1}$ (Jamieson et al., 2005); however, the concentrations of *E. coli* in the fecal material is 146-times greater than the average concentration of the seeded bottom sediments in the Jamieson study.

Table 1. Measurements of *E. coli* release and resuspension from a direct fecal deposit.

Downstream distance from cowpat (m)	Time after deposition (min)	Composite *E. coli* concentration (cfu 100 ml^{-1})	Resuspension or deposition rate (cfu $m^{-2}s^{-1}$)	1-hr cumulative load (cfu)	Attached fraction (%)
1.22	0.5	$1.81x10^4$	$5.91x10^7$	$5.75x10^7$	94.1%
1.22	15	$3.30x10^2$	$7.86x10^4$	$9.06x10^8$	91.6%
1.22	30	$3.13x10^2$	$7.46x10^4$	$9.37x10^8$	96.9%
1.22	60	$4.00x10^2$	$9.52x10^4$	$1.00x10^9$	77.2%
3.66	0.5	$4.83x10^4$	$2.19x10^6$	$1.54x10^8$	96.9%
3.66	15	$1.80x10^2$	$-1.09x10^4$	$2.39x10^9$	82.7%
3.66	30	$2.10x10^2$	$-7.49x10^3$	$2.41x10^9$	96.6%
3.66	60	$4.43x10^2$	$3.14x10^3$	$2.47x10^9$	99.5%

The attached fraction was not adjusted for background concentrations because it is unknown if the upstream contributions are particulate associated or in the unattached state. Attachment fractions ranged from 77.2 to 99.5%. Previous in-stream partitioning studies have identified attached fractions of fecal indicators as ranging between 20 and 35% during normal flow conditions and 30 to 55% during storms (Characklis et al., 2005; Krometis et al., 2007). Others have shown that up to 49% of *E. coli* released from cowpats during saturation-excess, sediment-laden flow attached to and are transported with manure colloids (Soupir et al., 2010). The nutrient rich environment of a fecal deposit encourages bacterial adhesion and flocculation, and the cells are released to the water column along with organic particulates.

Conclusions

Direct fecal deposits from cattle provide a significant source of pathogen indicators to streams, and reductions in direct fecal deposits are often the leading change in management practices necessary to meet water quality standards in agricultural watersheds. Experiments were conducted to assess the contribution of *E. coli* to the water column from a cowpat deposited into flowing water. At an average flow of 0.0106 m^3 s^{-1} and water depth of 15.24 cm, the cumulative load contribution from a single deposited cowpat after one hour was 2.49×10^9 cfu 3.66 m downstream. Between 77.2 and 99.5% of all *E. coli* downstream of the direct deposit were associated with particulates. The resuspension rate 0.5 min after deposition was 5.91×10^7 and 9.52×10^4 cfu $m^{-2}s^{-1}$ 60 minutes after deposition 1.22 m downstream of the deposit and 2.19×10^6 and 3.14×10^3 cfu $m^{-2}s^{-1}$ 0.5 min and 60 min after deposition, respectively. The composite *E. coli* concentrations all exceed federal standards for primary contact in the United States of 126 cfu/100 ml.

Results from this study are useful to improve modeling techniques to predict in-stream *E. coli* concentrations. New models can be developed from the experimentally determined time series release, resuspension and deposition of *E. coli* from direct fecal deposits as a function flow. Future experiments will compare the results of this study to experiments conducted with an additional water column depth and two additional flows. Further analysis of manure properties thought to influence in-stream processes including nutrient concentrations, manure particle size distribution, bulk density, and organic content are ongoing and will also be related to in-stream processes. Findings from this experiment emphasize the need to reduce cattle access to streams in areas impaired by high concentrations of fecal indicators. Options such as stream bank fencing or alternative water sources are recommended to reduce pollutant loadings to surface waters (Line, 2003); however, problems with fencing include wash-out during high flows and high maintenance. The lack of an alternative water source also limits the implementation of practices to reduce direct deposition. New management practices are needed to improve water quality in pastoral dominated watersheds.

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